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Granulocyte–Colony-Stimulating Factor After Allogeneic and Autologous Bone Marrow Transplantation in Children

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We evaluated the use of granulocyte CSF (G-CSF) after both allogeneic BMT (allo-BMT) and autologous BMT (ABMT) in children. After allo-BMT, G-CSF was used in 15 children who were compared with 20 historical controls. The ABMT patients were two sequential groups: the G-CSF group of 13 children and 11 historical controls. The patients were conditioned with different high-dose chemotherapy regimens with or without total body irradiation. G-CSF was administered at 5 μg/kg/day s.c. and was continued until an absolute neutrophil count (ANC) of 1,000 × 10^6/l was reached. Following allo-BMT, G-CSF accelerated myeloid engraftment with a difference of 5 days at the ANC level of 500 × 10^6/l ($P < 0.02$) and 9 days at 1,000 × 10^6/l ($P < 0.001$). In the ABMT patients, G-CSF also accelerated myeloid engraftment. The difference between the G-CSF group and the control group was 6 days at ANC 200 ($P < 0.05$), 11 days at ANC 500 ($P < 0.02$), and 17 days at ANC 1,000 ($P < 0.005$). In the ABMT patients, benefit by G-CSF was also observed in a smaller number of days with fever and days on antibiotics. We conclude that G-CSG significantly accelerated myeloid engraftment, after both allogeneic and autologous BMT in children, and also decreased the duration of febrile illness in the ABMT patients.

Key words: allogeneic bone marrow transplantation, autologous bone marrow transplantation, G-CSF, myeloid engraftment

INTRODUCTION

In the successful development of modern pediatric oncology, chemotherapy protocols are becoming more intensive for poor risk malignancies through both dose and frequency intensification. High-dose (HD) chemotherapy with bone marrow or peripheral blood stem cell rescue is also commonly used today. The major drawback of the HD therapies followed by allogeneic bone marrow transplantation (allo-BMT) or autologous bone marrow transplantation (ABMT) is the post-transplant period of profound neutropenia, rendering the patient susceptible to severe infections. The therapy of neutropenic sepsis is quite successful today [1,2], and even platelet refractoriness can be totally avoided in children by using filtered, leukocyte-free blood products [3,4], also allowing more liberal prophylactic use of blood components. Nevertheless, shortening of the post-transplant neutropenia is currently a focus of major research interest because it would potentially translate into decreased morbidity and cost. Myeloid growth factors are an attractive tool for this purpose.

The growth factors granulocyte–CSF (G-CSF) and granulocyte- macrophage–CSF (GM-CSF) are today widely used in BMT patients [5–8]. GM-CSF accelerates myeloid recovery after ABMT, which has been proven in several studies, including randomised ones [9,10–13]. Similar results have been achieved with G-CSF [14–17], although randomised studies are so far few [18–20]. Regarding allo-BMT, studies with growth factor use were initially delayed because there was concern about adverse effects in the form of graft- versus-host disease (GVHD) or graft failure. Subsequently these concerns have mostly been faded. GM-CSF has accelerated myeloid engraftment after allo-BMT [21–24], although less than after ABMT. G-CSF has been studied in the allo-BMT setting to a smaller extent; a dose-related acceleration of myeloid recovery has been observed [25–28]. The potential effect of G-CSF upon GVHD is still uncertain.

Most growth factor studies in BMT have been performed in adult patient series. Few pediatric studies have

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been published on the topic [29–32]. In general, the effects of CSFs in children appear to be similar to those in adults, and the growth factors have been very well tolerated by children [30,31,33,34]. The present study was designed to evaluate the use of nonglycosylated G-CSF in children after both ABMT and allo-BMT in a nonrandom, phase II setting, using historical controls for comparison.

PATIENTS AND METHODS

The G-CSF study was carried out as an open, single-institution pilot study at the Division of Pediatric Hematology-Oncology and BMT, Children's Hospital, University of Helsinki, Finland, during 1992–1994. In addition to institutional approval, oral witnessed informed consent (as is customary in Finland) was obtained from all parents/guardians of the patients participating in the study. The subjects belonged to two categories: the allo-BMT patients and the ABMT patients.

Allogeneic BMT Patients

In total 36 children underwent allo-BMT during the period 1988–1994. One patient with early toxic death did not survive long enough to demonstrate engraftment and is excluded from the analysis. Fifteen children received G-CSF, and 20 did not (control group).

The allogeneic bone marrows were harvested under general anesthesia by using the standard procedure. The attempted volume harvested was 10 ml of marrow per kilogram of recipient weight. Red cell depletion was performed in four cases for major ABO incompatibility by using the Haemonetics V50 system [35]. No other marrow ex vivo manipulations were attempted. The marrows from the Finnish Unrelated Donor Registry were harvested at the Third Department of Medicine, University of Helsinki, Finland, on the day of BMT by using a similar standard procedure, and were infused fresh and unmanipulated. GVHD prophylaxis consisted of cyclosporin A and a short course of methotrexate.

The nucleated cell dose of the marrow graft ranged from 0.7 to 7.4 $\times$ 10^8/kg. The mean $\pm$ standard deviation (SD) nucleated cell doses ($\times$ 10^8/kg of recipient weight) were 4.68 $\pm$1.77 in the G-CSF group and 3.72 $\pm$ 1.88 in the control group. The difference was not significant ($P < 0.20$). There was some correlation between the nucleated cell dose and myeloid engraftment when individual values of days of achieving an absolute neutrophil count (ANC) of 500 $\times$ 10^6/l were plotted against the nucleated cell dose per kilogram of recipient weight. We therefore also selected matched pairs from the patients, matching as closely as possible with the nucleated cell dose, with the age as the second priority, ending up with 11 patient pairs. Because we achieved similar results with the 11 matched pairs as compared with the whole data on 35 children, only data of the larger, noncensored material are presented.

TABLE I. Preparative Regimens in Allogeneic BMT Patients

	G-CSF	Controls
No XRT; Cy/Bu + Cy	3	1
Nodal XRT + Cy/Bu + Cy	2	3
TBI + Cy/Bu + Cy	7	9
TBI + HD-ARA-C	3	7
Total	15	20

XRT = irradiation; Cy = cyclophosphamide of 50 mg/kg/day $\times$ 4 days; Bu = busulfan of 4 mg/kg/day $\times$ 4 days; TBI = fractionated total body irradiation of 10–12 Gy; TBI + CY = TBI + cyclophosphamide of 60 mg/kg/day $\times$ 2 days; HD-ARA-C = cytosine arabinoside of 3 g/m^2/dose q 12 hours $\times$ 12, total 36 g/m^2.

G-CSF study group. Fifteen children, eight boys and seven girls, aged a median of 5.7 years (range, 2–16 years), transplanted during the period 1992–1994, were given G-CSF post-transplant. All consecutive BMT patients were included, except for those with myelogenous leukemias or mixed lineage acute lymphoblastic leukemia (ALL), to whom we decided not to give myeloid growth factor. The diagnoses were seven ALLs, two severe aplastic anemias, two severe combined immune deficiencies, one beta-thalassemia major, one Chediak-Higashi syndrome, one hemophagocytic lymphohistiocytosis, and one aspartylglucosaminuria. The preparative regimens, all marrow-ablative, are given in Table I. The marrow donors were matched siblings (n = 7), matched parental donors (n = 1), mismatched family members (n = 2), and matched unrelated donors from the Finnish national registry (n = 5).

Control group. The allo-BMT control group, in total 20 children, consisted of a historical group of all consecutive allo-BMT patients transplanted during 1988–1991 (n = 12), plus those with acute myeloblastic leukemia (AML), chronic myelocytic leukemia (CML) or mixed lineage ALL transplanted during the study period of 1992–1994 (n = 8). There were 11 boys and 9 girls, aged a median of 10.5 years (range, 1–17 years). The diagnoses were six ALLs, five acute nonlymphocytic leukemias (ANLL), three CMLs, five severe aplastic anemias, and one aspartylglucosaminuria. The preparative regimens, all marrow-ablative and very similar to those of the G-CSF study group, are given in Table I. The marrow donors were matched siblings (n = 15), matched parental donors (n = 2), mismatched family members (n = 1), and matched unrelated donors (n = 2).

ABMT Patients

The autologous bone marrows were harvested, frozen, and cryopreserved in liquid nitrogen 1 week to 20 months prior to the ABMT. The procedures were uniform and identical for all marrows. All marrows were tumor-free,

both by regular light microscopy and by using the indirect immunofluorescence technique with anti-GD2 monoclonal antibodies for neuroblastoma [36]. No ex vivo marrow purging procedures were attempted. Hematopoietic colony cultures were performed using test bags with small marrow volumes that were frozen separately with each autologous bone marrow. A standard procedure was utilized [37].

In total 45 children underwent ABMT during 1986–1994. Three patients with toxic deaths did not survive long enough to demonstrate engraftment and were excluded from the analysis. Eleven children received GM-CSF post-transplant and are not part of this analysis. The remaining 31 children were divided into the historical control group with no growth factor use (n = 17) and the G- CSF study group (n = 14).

The nucleated cell counts per recipient weight were all within the range of 0.9–3.0 × 10^8/kg. The mean ± SD nucleated cell counts (× 10^8/kg) were 1.85 ± 0.42 in the G-CSF study group and 1.49 ± 0.42 in the control group (n.s.). The correlation between the nucleated cell dose and myeloid engraftment was poor when the individual days of achieving an ANC of 500 × 10^6/l were plotted against the nucleated cell doses/kg. Therefore the nucleated cell doses were not taken into account in further analyses.

Because the dose of colony-forming units–granulocyte-macrophage (CFU-GM) is supposed to predict the time of myeloid engraftment even better than the nucleated cell dose, we also plotted the individual engraftment times in terms of ANC of ≥500 × 10^6/l against the CFU-GMs per kilogram of recipient weight (Fig. 1). There was a correlation at extremely low or extremely high CFU-GM doses only (Fig. 1). We decided to exclude from the analysis the ones with CFU-GMs of <2.0 × 10^4/kg (n = 2, Fig. 1), >10 × 10^4/kg (n = 3, Fig. 1), plus those (n = 2) who did not have the CFU-GM data available. After this exclusion procedure, the mean CFU-GM dose was 4.1 (range, 2.6–8.6) × 10^4/kg in the G-CSF group and 5.1 (range, 2.1–7.9) × 10^4/kg in the control group (n.s.). The censored ABMT material consisted of the following two sequential groups of patients.

G-CSF study group. This group included 13 children, 9 boys and 4 girls, aged a median of 4.7 years (range, 1–15 years), transplanted during 1992–1994. The diagnoses were four poor-risk neuroblastomas, two soft tissue sarcomas, three Wilms' tumors, two germ cell tumors, one Ki-1 lymphoma, and one desmoplastic abdominal tumor.

Control group. This group with no growth factor use included 11 children, 7 boys and 4 girls, aged a median of 7.7 years (range, 1–17 years), transplanted during 1987–1992. The diagnoses were as follows: four poor risk neuroblastomas, one peripheral neuroepithelial tumor, one Ewing's sarcoma, one high-grade glioma, two Wilms' tumors, one histiocytic lymphoma, and one ALL. The preparative regimens for the ABMT patient groups are given in Table II. All regimens were marrow-ablative.

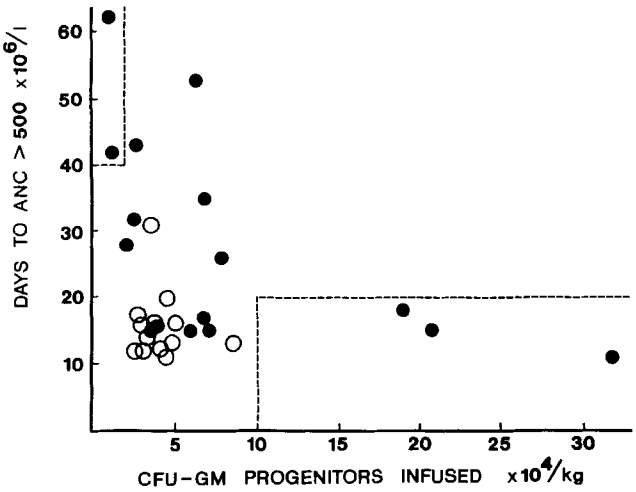

Fig. 1. Correlation of myeloid engraftment with the CFU-GM dose in pediatric ABMT patients. Individual myeloid engraftment times in terms of days of achieving the ANC level of 500 × 10^6/l are plotted against the dose of CFU-GM progenitors/kg infused. Open circles: G-CSF group patients (n = 13). Solid circles: historical controls with no growth factor use (n = 16). The dotted lines indicate exclusion of individuals with very low or very high CFU-GM doses.

TABLE II. Preparative Regimens in ABMT Patients

	G-CSF	Controls
HD-thiotepa	2	4
HD-thiotepa + carbo + VP-16	3	—
HD-melphalan	4	1
TBI + VMP	4	4
TBI + Cy/Bu + Cy	—	2
Total	13	11

HD-thiotepa = thiotepa of 375 mg/m²/day × 3 days; HD-thiotepa + carbo + VP-16 = thiotepa of 300 mg/m²/day × 3 days, carboplatinum of 500 mg/m²/day × 2 days, plus VP-16 of 700 mg/m²/day × 2 days; HD-melphalan = melphalan of 210 mg/m²; TBI = fractionated total body irradiation of 10–12 Gy; VMP = cisplatinum of 90 mg/m² on day -9, VP-16 of 150 mg/m² on days -7 and -4, melphalan of 140 mg/m² on day -6 and 70 mg/m² on day -5; TBI + Cy = TBI + cyclophosphamide of 60 mg/kg/day × 2 days; Bu + Cy = busulfan of 4 mg/kg/day × 4 days, cyclophosphamide of 50 mg/kg/day × 4 days.

Administration of G-CSF

Recombinant human G-CSF was used in a nonglycosylated form (Neupogen®, Roche Pharmaceuticals, Basel, Switzerland), at a dosage of 5 μg/kg/day subcutaneously, started on day +1 post-transplant in both allo-BMT and ABMT patients, and was continued daily until the ANC exceeded 1000 × 10^6/l.

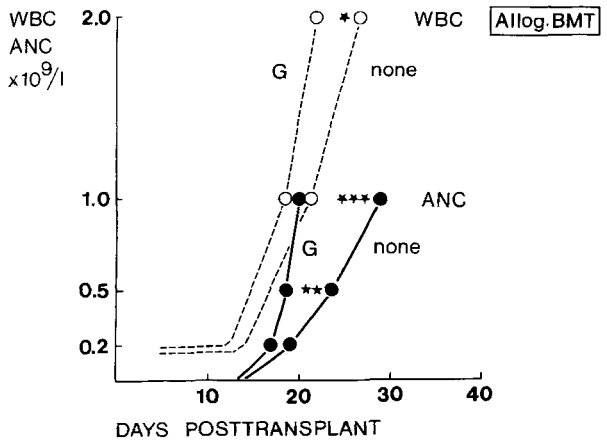

Fig. 2. Myeloid engraftment in pediatric allogeneic BMT recipients. The patients either received G-CSF until an ANC of 1,000 × 10^6/l (G; n = 15) or no growth factor (none; n = 20). The dotted lines indicate WBC mean values, and the solid lines indicate ANC mean values. The asterisks indicate significant differences: *$P < 0.05$, **$P < 0.02$, ***$P < 0.001$.

Statistical Methods

Student's t-test for two independent samples and the nonparametric median test were used in the statistical analyses.

RESULTS

Allogeneic BMT Patients

The use of G-CSF facilitated myeloid engraftment. The difference between the G-CSF group and the control group was 2 days at the ANC level of 200 × 10^6/l (n.s.), 5 days at an ANC of 500 × 10^6/l ($P < 0.02$), and 9 days at an ANC of 1,000 × 10^6/l ($P < 0.001$) (Fig. 2). The total WBC count of 1.0 × 10^9/l was achieved 3 days earlier (n.s.), and WBC of 2.0 was achieved 7 days earlier ($P < 0.05$) in the G-CSF group (Fig. 2).

It is remarkable, however, that while all patients in the historical control group engrafted within the normal time, we had one case of early graft failure in the G-CSF group. On day +26 he still had zero level of circulating granulocytes and a totally empty bone marrow, as evaluated in the BM aspirate and biopsy samples. His myeloid engraftment occurred after transfusions with irradiated leukocytes for a life-threatening infection, with an ANC of 200 × 10^6/l on day +34, 500 × 10^6/l on day +41, and 1,000 × 10^6/l on day +45 [38]. Daily G-CSF was continued until an ANC of 1,000 × 10^6/l, per protocol. Cytogenetic evidence of allogeneic engraftment was obtained (female donor, male recipient). This patient is included, as the only leukocyte transfusion recipient, in the G-CSF study group data analysed (and was even included in the matched pairs mentioned in the Patients and Methods).

There was no difference in platelet engraftment. The platelets were self- sustained at the level of 30 × 10^9/l at a median of 4 weeks in both patient groups. Late graft failures were not observed in the G-CSF group, but occurred in 2 of 20 in the control group. One three-lineage graft failure (with one-locus mismatch sibling donor) occurred at about 100 days post-transplant but responded well to interleukin-3 (IL-3) therapy. The other graft failure (HLA-matched maternal graft) was 8 months post-transplant in connection with severe chronic GVHD and a fulminant CMV infection, in which the patient succumbed.

The incidence of severe infections was similar in both groups. Documented septicemia was seen in 6 of 15 in the G-CSF group, and in 7 of 20 in the control group. All children had febrile neutropenia post-transplant and received broad-coverage antibiotics. The mean number ± SD of days with fever was 9.5 ± 6.8 days in the G-CSF group and 7.8 ± 10.9 in the control group (n.s.). The mean number ± SD of days on antibiotics was also similar: 21.9 ± 10.3 days in the G-CSF group and 24.0 ± 16.8 days in the control group (n.s.). The duration of hospital stay had a very wide range of 25–102 days, with a median duration of 50 days (range, 27–102 days) in the G-CSF group and of 35 days (range, 25–100 days) in the control group (n.s.).

G-CSF did not increase the incidence or severity of acute GVHD. In the G-CSF group, grade III–IV acute GVHD was observed in 3 of 15 (20%) of all BMT patients, and in 0 of 7 of those with matched sibling donors. The corresponding figures in the control group were 4 of 20 (20%) for all BMT patients, and 2 of 15 (13%) for those with matched sibling donors. Leukemia relapses post-transplant were not more frequent in the G-CSF group, although the numbers are very small and AMLs were not included. Of evaluable ALL patients who received allografts, 2 of 7 relapsed in the G-CSF group, compared with 2 of 4 in the control group.

ABMT Patients

The use of G-CSF clearly facilitated myeloid engraftment. The difference between the G-CSF group and the control group with no growth factor use was 6 days at ANC of 200 × 10^6/l ($P < 0.05$), 11 days at ANC of 500 × 10^6/l ($P < 0.02$), and 17 days at ANC of 1,000 × 10^6/l ($P < 0.005$) (Fig. 3). The total WBC of 1.0 × 10^9/l was achieved 5 days earlier ($P < 0.05$) and the WBC of 2.0 × 10^9/l was achieved 10 days earlier ($P < 0.01$) in the G-CSF group as compared with the control group (Fig. 3).

Platelet recovery was not facilitated by G-CSF. The platelets were self- sustained at the level of 30 × 10^9/l in the G-CSF group at a median of 6 weeks (range, 2.5–24 weeks), and in the control group at 8 weeks (range, 3–16 weeks) (n.s.). No difference was recognized in the inci-

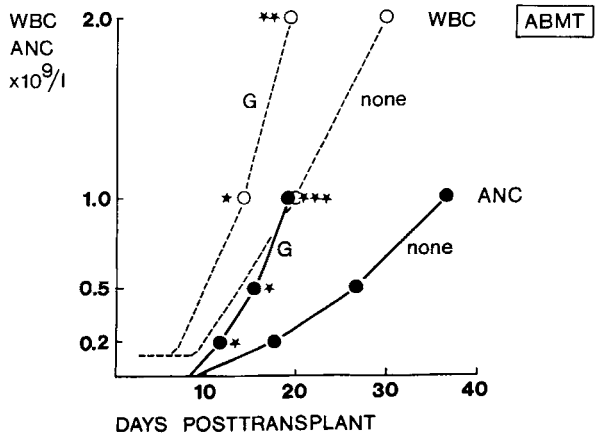

Fig. 3. Myeloid engraftment in pediatric ABMT patients. The children either received G-CSF until an ANC of 1,000 × 10^6/l (G; n = 13) or no growth factor (none; n = 11). The dotted lines indicate WBC mean values, and the solid lines indicate ANC mean values. The asterisks indicate significant differences: *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$.

dence of severe infections between the groups. Documented septicemia was seen in 3 of 13 in the G-CSF group and in 1 of 11 in the control group. All children had febrile neutropenia post-transplant and were treated with broad-coverage antibiotics. The mean number ± SD of days with fever was 6.5 ± 4.9 days in the G-CSF group, compared with 13.0 ± 7.3 days in the control group ($P < 0.02$). The mean number ± SD of days on antibiotics was 15.8 ± 5.4 days in the G-CSF group and 21.4 ± 8.2 days in the control group ($P < 0.10$). The median duration of hospital stay was 27 days (range, 21–45 days, plus one patient for 95 days) in the G-CSF group, and 37 days (range, 21–50 days) (n.s.) in the control group.

Tolerability of G-CSF

With the dosage and s.c. route used, the daily G-CSF administration was very well tolerated in all children. No adverse effects were seen. Specifically, no rashes, fevers, myalgias, bone pains, or significant fluid retentions attributable to G-CSF were observed.

DISCUSSION

This is among the first pediatric studies that evaluate the use of G-CSF in ABMT and allo-BMT patients. Our study groups were formed sequentially, so the data need to be interpreted with the nonrandomized pilot study character in mind. Our main finding is that G-CSF accelerated myeloid engraftment in children after both allogeneic and autologous BMT.

In the allograft patients receiving G-CSF, the ANC levels of 200, 500, and 1,000 × 10^6/l were achieved on the mean post-transplant days of +17, +18, and +20, respectively, which were 2 days, 5 days ($P < 0.02$), and 9 days ($P < 0.001$) earlier than in the control group (Fig. 2). If the typical time to recovery of 500 neutrophils is 25 days [7], even our control group engrafted relatively fast (ANC of 500 × 10^6/l in 23 days). Platelet engraftment was not influenced by G-CSF; other reports of G-CSF use in allografts do not give platelet data [25–28]. Growth factors, particularly GM-CSF, have been successfully used in graft failure patients [39]. Our experience is, in contrast, one patient who had primary graft failure despite the use of G-CSF; he reached an ANC of 500 × 10^6/l as late as day +41. He engrafted subsequently well, although slowly. In reference to this patient, we believe that several other cytokines, in addition to G-CSF, are required for engraftment [38]. G-CSF did not increase the incidence or severity of acute GVHD, and G-CSF use was not associated with late graft failures, in accord with findings in adult studies [25,26,28].

In the ABMT patients, myeloid engraftment was substantially accelerated by G-CSF. Post-transplant days for the ANC levels of 200, 500, and 1,000 × 10^6/l were +11, +14, and +19, respectively, being as great as 6 days ($P < 0.05$), 11 days ($P < 0.02$), and 17 days ($P < 0.005$) earlier than in the historical control group (Fig. 3). In the control group the 25 days until an ANC of 500 should again correspond to a common average [7]. Platelet recovery was not benefitted by G-CSF, in accord with reports in adults [14,15,17,18].

The incidence of severe infections, the number of days with fever, days on antibiotics, and the duration of hospital stay were not reduced by the use of G-CSF in the allograft recipients. On the other hand, in the ABMT patients a benefit from G-CSF was observed in the reduction of the number of days with fever (6 vs. 13 in the controls; $P < 0.02$), and possibly of days on antibiotics (16 vs. 21 in the controls; $P < 0.10$). Apparently the duration of the absolute ANC nadir is not influenced by the use of G-CSF in either allo-BMT or ABMT patients, with the incidence of severe infections being consequently unaffected. Faster myeloid engraftment seems, however, to translate into somewhat reduced post-transplant morbidity. In adult studies the role of G-CSF in infectious morbidity has been variable. Reduction of the number of febrile days or days on antibiotics has been observed in most [14,15,17,20,28] but not all [18] studies.

G-CSF was very well tolerated in children at the dose of 5 μg/kg/day. In our previous study with GM-CSF utilizing the same dose, mild adverse effects were observed, including skin rash, urticaria, itching, stuffy nose, mild edema, and general achiness [31]. In the present G-CSF series no adverse effects were observed.

In the future, combinations or sequential use of cytokines, for example, IL-3 followed by GM-CSF is likely to emerge instead of using a single growth factor. The sequential use of GM-CSF and G-CSF has already shown some promise [32]. Currently an alternative approach to achieve an earlier myeloid engraftment is the use of pe-

ripheral blood stem cell (PBSC) transplants, either alone or in combination with ABMT, with or without additional post-transplant CSF use. In small-size pediatric patients, the collection of PBSC by apheresis may still offer some technical problems. The PBSC collections have not yet been widely applied to allogeneic stem cell donations.

In conclusion, G-CSF accelerated myeloid engraftment significantly after both allogeneic and autologous BMT in children, and also decreased the duration of post-transplant febrile illness in the ABMT patients. Platelet engraftment was not influenced. G-CSF was extremely well tolerated. The use of G-CSF is of benefit in children after both allogeneic and autologous BMT.

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